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Tracking no: ADV-2022-007751R1

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Abstract:
Immune thrombocytopenia (ITP) is an autoimmune bleeding disorder with an incompletely understood pathophysiology, but includes platelet-clearance in the spleen and liver via T-cells and/or platelet-autoantibodies. Strikingly, thrombopoietin (TPO) levels remain low in ITP. Platelet-glycoprotein (GP)Ibα has been described to be required for hepatic TPO generation, however, the role of GPIb-antibodies in relation to platelet hepatic sequestration and TPO-levels, with consideration of platelet counts, remains to be elucidated. Therefore, we performed a study in which we included 53 chronic and non-splenectomized ITP patients for which we conducted indium labeled autologous platelet scintigraphy, measured platelet-antibody profiles and TPO-levels. Upon stratification towards the severity of thrombocytopenia, no negative association was observed between GPIb/IX-antibodies and TPO levels, suggesting that GPIb/IX-antibodies do not inhibit or block TPO levels. Surprisingly, we observed a positive association between GPIb/IX-antibody levels and TPO levels, and GPIb/IX-antibodies and platelet hepatic sequestration, in patients with severe thrombocytopenia, but not in patients with mild or moderate thrombocytopenia. In addition, platelet hepatic sequestration and TPO levels were positively associated. This collectively indicates that GPIb/IX-antibodies may be associated with an increased platelet hepatic sequestration and elevated TPO levels in severe thrombocytopenic ITP patients, however, further research is warranted to elucidate the pathophysiological mechanisms.

Conflict of interest: No COI declared

COI notes:

Preprint server: No;

Author contributions and disclosures: RK, SNA, VSN and MS conceived and designed the study; SNA and RK performed the study and analyzed data, SNA and RK wrote the manuscript; and VSN, LP, TN, JJZ, MdH and MS critically reviewed and edited the manuscript. All authors interpreted the data and approved the final draft.

Non-author contributions and disclosures: No;

Agreement to Share Publication-Related Data and Data Sharing Statement: Data sharing is possible, feel free to email the corresponding author.

Clinical trial registration information (if any):

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Running title: Interplay GPIb/IX antibodies, liver, and TPO in ITP

Data sharing: Contact the corresponding author for data sharing: r.kapur@sanquin.nl.

Counts

Abstract word count: 199

Text word count: 1175

Number of figures: 1

Number of tables: 1 , supplemental tables: 2

Reference count: 16

Abstract

Immune thrombocytopenia (ITP) is an autoimmune bleeding disorder with an incompletely understood pathophysiology, but includes platelet-clearance in the spleen and liver via T-cells and/or platelet-autoantibodies. Strikingly, thrombopoietin (TPO) levels remain low in ITP. Platelet-glycoprotein (GP)Ibα has been described to be required for hepatic TPO generation, however, the role of GPIb-antibodies in relation to platelet hepatic sequestration and TPO-levels, with consideration of platelet counts, remains to be elucidated. Therefore, we performed a study in which we included 53 chronic and non-splenectomized ITP patients for which we conducted indium labeled autologous platelet scintigraphy, measured platelet-antibody profiles and TPO-levels. Upon stratification towards the severity of thrombocytopenia, no negative association was observed between GPIb/IX-antibodies and TPO levels, suggesting that GPIb/IX-antibodies do not inhibit or block TPO levels. Surprisingly, we observed a positive association between GPIb/IX-antibody levels and TPO levels, and GPIb/IX-antibodies and platelet hepatic sequestration, in patients with severe thrombocytopenia, but not in patients with mild or moderate thrombocytopenia. In addition, platelet hepatic sequestration and TPO levels were positively associated. This collectively indicates that GPIb/IX-antibodies may be associated with an increased platelet hepatic sequestration and elevated TPO levels in severe thrombocytopenic ITP patients, however, further research is warranted to elucidate the pathophysiological mechanisms.

Key Points

- GPIb/IX-antibodies do not appear to inhibit or block TPO production in ITP patients when stratified towards the degree of thrombocytopenia.
- GPIb/IX-antibodies may be associated with higher TPO and increased platelet hepatic sequestration under severe thrombocytopenic conditions.

Introduction

Immune thrombocytopenia (ITP) is an autoimmune bleeding disorder characterized by low platelet counts (<100 x 10^9 / L).[1] The pathophysiological pathways of platelet clearance in ITP are not yet fully unraveled, but involve T cells and/or platelet autoantibodies.[2] One of the most investigated pathways of platelet clearance include the effects of autoantibodies directed against glycoprotein (GP) complexes.[3] Autoantibody binding to GP complexes, present on the platelet membrane, can lead to liver and/or spleen sequestration, phagocytosis and subsequently to thrombocytopenia.[2] This predominantly occurs via antibody-Fc mediated recognition by Fcγ-receptors on macrophages, resulting in phagocytosis in spleen and/or liver.[4] There is, however, evidence that Fc-independent mechanisms of ITP also exist, leading to platelet hepatic sequestration.[5]

Thrombopoietin (TPO) regulates platelet production via interaction with myeloproliferative leukemia protein receptor (Mpl; CD110) which is present on megakaryocytes and circulating platelets.[6] TPO plasma levels are mainly derived from the active and continuous TPO production by the liver and to a lesser extent by the spleen, kidney and bone marrow.[6] In addition, TPO production is induced by the binding of desialylated aged platelets through interaction with the hepatic Ashwell-Morrell receptor (AMR).[6] Furthermore, it has been shown that certain GPIbα-antibodies trigger platelet desialylation, a process that additionally increases the clearance of these platelets via the hepatic AMR.[7] In ITP patients, remarkably, TPO levels remain relatively low. GPIbα, independently of platelet desialylation, was demonstrated to be required for hepatic TPO generation in mice.[8] GPIbα -/- mice showed lower TPO levels compared to wildtype mice, and in agreement, patients with Bernard-Soulier Syndrome (BSS), who lack the GPIb-IX-V complex, have low TPO levels with low to moderate platelet counts.[8] In that respect, it was suggested that GPIb/IX-antibodies may interfere with hepatic TPO production in ITP, possibly explaining the relatively low TPO levels in patients with ITP.[8] A study by Porcelijn et al, however, did not find an association between GPIb/IX-antibodies and TPO levels in a large cohort of 3490 patients suffering from ITP.[9] This study did not incorporate data on platelet counts of these patients. The latter could be of importance because in healthy subjects it

is well known that, apart from the via AMR-binding induced TPO production, the total platelet mass negatively influences the unbound and measurable TPO levels.[10] As low platelet counts in ITP patients do not intriguingly trigger high TPO levels, we aimed to shed light on the interplay between GPIb/IX-platelet antibodies, the site of platelet sequestration, and TPO levels, with consideration of platelet counts in a cohort of chronic and non-splenectomized ITP patients.

Methods

In this study we investigated both the association between 1) GPIb/IX-antibodies and the site of platelet sequestration by indium labeled autologous platelet scintigraphy and 2) GPIb/IX antibodies and TPO levels in a cohort of 53 chronic and non-splenectomized ITP patients.[11] The included patients had a clinical indication for a scintigraphy scan as indicated by the treating hematologist (indication for a second/third line therapy with splenectomy as one of the therapeutic options), and had a mean age at diagnosis of 36 (SD ± 18) years. Importantly, we stratified these patients by the degree of thrombocytopenia: mild (platelet counts >50 x 10^9 / L) and moderate/severe (<50 x 10^9 / L). An additional sensitivity analysis was performed for severe thrombocytopenia (platelet count <25 x 10^9/L). Antibody levels were measured using direct (antibody bound directly on patient platelets) and indirect (antibody binding on donor platelets incubated with serum from the patient) Monoclonal Antibody-specific Immobilization of Platelet Antigen (MAIPA), with a cut-off of 0.130 OD (optical density).[12] TPO levels were measured as described by Folman et al. with a normal range in healthy subjects of 4-32 A.U./ml.[13] All patients underwent an Indium-111 labeled autologous platelet sequestration scintigraphy; the sequestration outcome was used as a continuous variable ranging 0 to 100% sequestration in liver. In the clinical setting the outcome is categorized in splenic, mixed and platelet hepatic sequestration pattern based on the splenic to liver ratio (S:L ratio).[14] Associations between TPO levels and anti-GP antibody levels were primarily tested using linear regression models, and multivariable models included platelet counts as a confounder. Differences were considered

statistically significant at $p < 0.05$. The study was approved by the Dutch Medical Ethical Review Board, which was conducted in accordance with the Declaration of Helsinki.

Results and discussion

Anti-platelet antibodies were measured in 53 patients. 29 patients had mild thrombocytopenia (platelet count >50 $x10^9$/L) and 24 patients had moderate to severe thrombocytopenia (platelet count <50 $x10^9$/L). Of the latter group 6 patients had severe thrombocytopenia (plt <25 $x10^9$/L). GPIb/IX-antibody OD values were found to be above the detection threshold in 13 of these patients (direct MAIPA, and 11 patients using the indirect MAIPA), of which 9 patients suffered from mild and 4 patients from moderate/severe thrombocytopenia. The presence of other platelet-antibodies, using direct and indirect MAIPA, in these 13 patients is depicted in Table S1. Upon stratification towards the severity of thrombocytopenia, no negative association was observed between GPIb/IX-antibodies (direct and indirect MAIPA) and TPO levels (Table 1). This suggests that GPIb/IX-antibodies levels do not inhibit or block the regulation of TPO levels in ITP patients, indicating that other factors and pathways are responsible for the relatively low levels of TPO in patients with ITP. Surprisingly, a significant positive association was observed between GPIb/IX-antibody levels (direct MAIPA) and TPO levels, and GPIb/IX-antibodies (direct and indirect MAIPA) and platelet hepatic sequestration, in patients with severe thrombocytopenia, but not in patients with mild (direct and indirect MAIPA) or moderate thrombocytopenia (direct MAIPA) (Table 1). In addition, platelet hepatic sequestration and TPO levels were positively associated (Table 1). Collectively, this may suggest that GPIb/IX-antibodies could be associated with an increased platelet hepatic sequestration and elevated TPO levels in severe thrombocytopenic ITP patients (by a direct and/or 2 sequentially indirect pathways as indicated in Figure 1). In contrast, we found no significant associations between GPIIb/IIIa- or GPV-antibodies (direct and indirect MAIPA) and platelet hepatic sequestration or TPO levels in ITP patients with severe thrombocytopenia (Table S2). This indicates that the previously mentioned associations may be specific for GPIb/IX-antibodies under severe thrombocytopenic conditions.

In this study we find that GPIb/IX-antibodies do not appear to inhibit or block TPO production in ITP patients when stratified towards the degree of thrombocytopenia. In addition, we find that GPIb/IX-antibodies may be associated with a stimulated TPO production through the liver, but only under severe thrombocytopenic conditions. In this setting of increased clearance, there may be an additional contribution of specific GPIb/IX-antibodies directed against the ligand-binding domain (LBD) in a Fc-independent manner.[15] This may result in mechanomolecular signaling[15] leading to increased platelet clearance via the liver which may stimulate an increase in TPO levels through a feedback mechanism. Although GPIbα was suggested to be required for hepatic TPO generation independently of platelet desialylation in mice[8], our human data suggests that under severe thrombocytopenic conditions there may be an additional contribution of GPIb/IX-induced platelet-desialylation and consequently increased hepatic clearance via the AMR, resulting in increased TPO levels. For the first time, our results support a possible link between GPIb/IX-antibodies, platelet hepatic sequestration and increased TPO levels in ITP patients with platelet counts below 25 $x10^9$/L. Although suggestive, from observational data we cannot conclude on causality. Possible bias may be introduced through indication of scanning relapse and refractory patients. Furthermore, it has been described that different types of GPIb-antibodies can have divergent functions [16], however, in the current study we were unable to differentiate the different types of GPIb-antibodies. Therefore, the mechanistic pathways should be further experimentally explored in vitro and in vivo animal models. Importantly, these results will also need to be validated in larger cohorts. The current findings add to the largely unknown pathophysiology of ITP and could ultimately be applied for the development of new individualized treatment options for patients suffering from ITP.

Authorship contributions:

RK, SNA, VSN and MS conceived and designed the study; SNA and RK performed the study and analyzed data, SNA and RK wrote the manuscript; and VSN, LP, TN, JJZ, MdH and MS critically reviewed and edited the manuscript. All authors interpreted the data and approved the final draft.

Disclosure of Conflicts of Interest:

All authors declare no conflict of interest.

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Tables

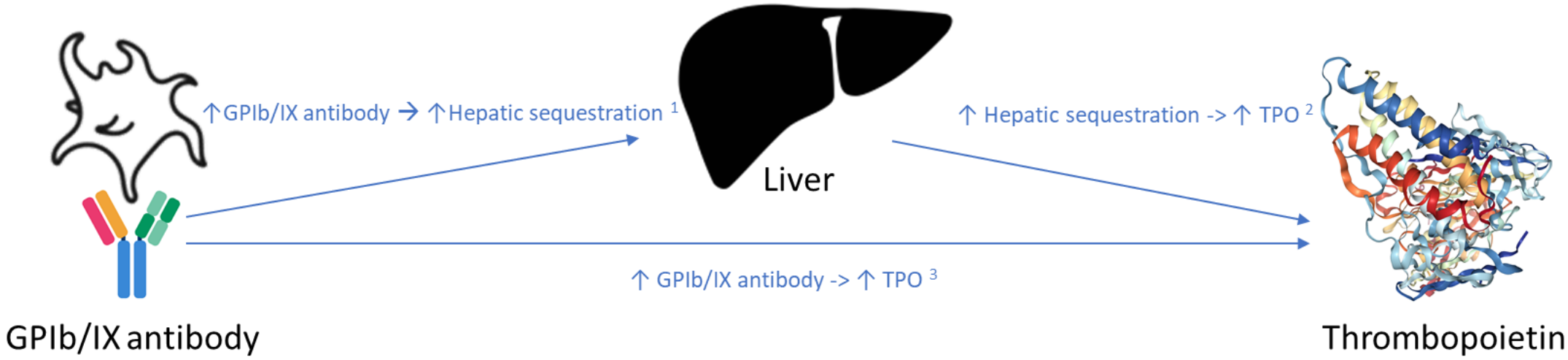
Table 1: Regression models for the association between anti GPIb/IX antibodies, TPO levels and platelet hepatic sequestration, under thrombocytopenic conditions, in a cohort of chronic and non-splenectomized ITP patients. Statistical significance: $p < 0.05$ (*).

	Linear regression ITP patients with platelet count >50 x 10^9/L (n=29) β (95%-CI), p value	Linear regression ITP patients with platelet count <50 x 10^9/L (n=24) β (95%-CI), p value	Sensitivity analysis: Linear regression ITP patients with platelet count <25 x 10^9/L (n=6) β (95%-CI), p value
Association 1: Anti-GPIb/IX & TPO-level	**Direct** MAIPA: 0.000 (-0.002 – 0.002), p=0.91 **Indirect** MAIPA: 0.000 (-0.0005 – 0.004); p=0.88	**Direct** MAIPA: -0.045 (-0.180 – 0.089); p=0.489 **Indirect** MAIPA: 0.051 (0.004 – 0.097) p= 0.03*	**Direct** MAIPA: 0.092 (0.012-0.172), p=0.03* **Indirect** MAIPA: 0.116 (-0.131 – 0.364) p=0.18
Association 2: Anti-GPIb/IX & platelet hepatic sequestration	**Direct** MAIPA: -0.001 (-0.004 – 0.002), p=0.63 **Indirect** MAIPA: -0.002 (-0.004 – 0.001) p=0.22	**Direct** MAIPA: 0.025 (-0.029 – 0.079), p=0.35 **Indirect** MAIPA: 0.020 (0.006 – 0.035) p=0.008*	**Direct** MAIPA: 0.026 (0.006 – 0.045), p=0.02* **Indirect** MAIPA: 0.045 (0.003 – 0.088) p=0.04*
Association 3: TPO-level & platelet hepatic sequestration	-0.076 (-0.793 – 0.641) p=0.83	0.262 (0.124 – 0.400) p=0.001*	0.228 (CI 0.126 – 0.331), p=0.002*

Figure legend

Interplay between GPIb/IX antibodies, platelet hepatic sequestration and TPO levels in chronic and non-splenectomized ITP patients with severe thrombocytopenia.

Figure 1



Footnote:
[1] Association found between GPIb/IX antibody levels and increased hepatic sequestration of platelets.
[2] Association found between hepatic sequestration and increased TPO levels.
[3] Direct association between GPIb/IX antibody levels and increased TPO levels.